Abstract No. Silv0054

Vandium Compounds as Novel Inhibitors of the R61 D-ala-D-ala Peptidase

N. Silvaggi, G. Fuhrer, J. Anderson, and J. Kelly (Univ. of Connecticut)

Beamline(s): X12B, X4A

Introduction: Most species of bacteria synthesize and maintain a rigid cell wall. The D-alanyl-D-alanine carboxypeptidase/transpeptidases (DD-peptidases) are crucial components of the cell wall synthetic machinery. These enzymes are responsible for the cross-linking of neighboring peptidoglycan strands in the final step of bacterial cell wall biosynthesis. Because β-lactam antibiotics mimic the natural D-ala-D-ala substrate of the DD-peptidases, these enzymes are the targets of β-lactams such as penicillins and cephalosporins. These drugs inhibit DD-peptidases by forming very long-lived acyl enzyme intermediates. The DD-peptidase from *Streptomyces* strain R61, a 37.5kDa exocellular enzyme, has served extensively as a model for the membrane-bound DD-peptidases that catalyze the majority of cell wall cross-linking.

In response to the widespread use of β-lactam antibiotics, bacterial resistance to these drugs is growing rapidly, primarily through bacterial production of β-lactamases. β-Lactamases are enzymes that react with β-lactam compounds, opening the lactam ring and rendering the drugs useless against their intended targets, the DD-peptidases. Unlike DD-peptidases, β-lactamases form very short-lived acyl enzyme intermediates with penicillins and cephalosporins. The existence of β-lactamase mediated resistance to current antibiotics threatens the continued efficacy of β-lactams as therapeutic agents. Thus there is an urgent need to develop non-β-lactam inhibitors of DD-peptidases for use against infectious diseases.

Often good inhibitors mimic transition states in an enzymatic reaction. For example, phosphonate compounds are very effective inhibitors of DD-peptidases, and they have been shown crystallographically to mimic the tetrahedral transition state in the reaction of these enzymes with normal substrate. Thus, a compound that goes one step further and mimics the pentacoordinated transition state of the phosphonate reaction could be a new class of DD-peptidase inhibitor. Kinetic studies by Dr. R.F. Pratt and coworkers at Wesleyan University have shown that vanadate (VO_4^-) and hydroxamic acids, in 1:1 complexes, are potent DD-peptidase inhibitors. Vanadium is capable of forming a pentacoordinated complex that could mimic the phosphonate transition state. We are in the process of determining the structures of the R61 DD-peptidase complexed with vanadate: hydroxamate inhibitors in order to learn more about how these compounds interact with the enzyme.

Results: Extensive crystallographic studies have been performed on the R61 DD-peptidase in complex with a variety of β-lactam inhibitors, as well as a peptide substrate. We have recently determined the structures of the non-covalent Henri-Michaelis peptide complex (R=0.15 with R_{free}=0.18, 1.9Å resolution), a non-covalent enzyme-products complex (R=0.11, R_{free}=0.15, 1.2Å), and a covalent complex with a phosphonate peptide that we interpret as a tetrahedral transition state analog (R=0.11, R_{free}=0.14, 1.1Å). Preliminary structures of two vanadate: hydroxamate complexes (VO_4^-: p-NO_2 benzohydroxamic acid and VO_4^-: m-MeO benzohydroxamic acid) show that the inhibitors both appear to adopt pentacoordinate geometries with the catalytic Ser62 OG acting as one of the ligands. However, neither compound interacts very well with other residues in the enzyme active site. The p-NO_2 compound actually makes no contacts with the active site other than the covalent linkage to the catalytic serine OG. The m-MeO compound does not bind particularly well as evidenced by multiple conformations, however it does take on a conformation similar to that seen with some β-lactams, with the aromatic ring interacting with a small hydrophobic surface at the back of the active site pocket. All the ternary complex structures (R61: VO_4^-: hydroxamic acid) obtained to date have been complicated by the fact that key catalytic residues in the enzyme active site have been chemically cross-linked to varying extents. The cross-linking occurs as a result of formaldehyde in the crystallization media, and work is currently in progress to eliminate the cross-linking in order to obtain the highest occupancy possible of the vanadium complexes. A clear structure of a ternary complex is highly desirable since it could provide insight into how the enzyme manages to stabilize the pentacoordinated vanadate complex, when molecular modeling studies have suggested that the active site would not accommodate such structures easily. Knowledge of the detailed interactions of intermediates along the reaction pathway will inform efforts to design more effective antibacterial agents.